Distribution of HLA class 1 antigens in normal human tissue and in mammary cancer

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SUMMARY With a monoclonal antibody which reacts with all HLA class 1 antigens it was found that these antigens are not uniformly distributed in all nucleated cells. Rather HLA class 1 antigens are restricted in their distribution to lymphoid cells, endothelial cells of small vessels, and certain epithelia including mammary duct cells. These antigens were not detected on hepatocytes, specialised cells of the central nervous system, or on the tumour cells of 8 out of 17 human mammary cancers. Given the hypothesis that T cells only respond to foreign antigens on cells which share a common major histocompatibility antigen, these results imply that the T cell responses to viral infections of hepatocytes—for example, hepatitis B virus and the CNS—for example, subacute sclerosing encephalitis, are mediated through an antigen system other than HLA class 1. The absence of HLA class 1 antigen on many mammary cancer cells may be of prognostic significance if T cell modulation of tumour growth is mediated through this class of antigens.

HLA antigens are human cell surface glycoproteins. They are of two types, class 1 (HLA-A, B, C) and class 2 (HLA-DR).¹ Evidence from absorption studies of anti-HLA antisera by tissue homogenates² indicates that HLA class 1 antigens are present on virtually all nucleated cells.^{2,3} With the exception of the thymus⁴ and kidney³ however, there are few direct demonstrations of HLA antigens on the surface of cells in intact tissues, mainly due to lack of appropriate reagents.

HLA antigens appear to play a role in regulating many immune responses including those involved in the discrimination between self and non-self.^{5,6} One way in which these antigens regulate immune responses is by major histocompatibility complex (MHC) restriction of T cell mediated immunity.⁷ This mechanism implies that a T cell will only respond to a target antigen on a cell surface if that cell also carries at least one major histocompatibility antigen in common with the T cell. This applies not only to immune responses to viral infections^{8,9} but possibly also to T cell-mediated responses to tumour

cells.¹⁰ The latter is exemplified by the absence of T cell cytotoxicity against embryonal carcinomal cells which do not express MHC antigens on their cell surface.¹¹ There is also evidence that murine tumour growth can be modulated in vivo and in vitro by immune reactions, especially T cell cytotoxicity.¹²

Our study examines two problems: the distribution of HLA class 1 antigens in intact normal tissues and the in situ expression of these antigens in human breast carcinomas. Using a monoclonal antibody directed against all HLA class 1 antigens it is shown by immunofluorescence that these antigens in normal tissues are detectable only in the lining cells of small blood vessels, lymphoid cells and a restricted number of epithelia including mammary ductal epithelium; by contrast the expression of HLA-A, B, C antigens by malignant ductal epithelium varies from tumour to tumour.

Material and methods

The mouse monoclonal antibody (PA 2·6) to HLA class 1 antigens has been described previously.¹³ In brief, spleen cells from a mouse immunised with papain solubilised, purified HLA antigens were fused

with mouse myeloma cell line NS1. One of the resulting hybrid clones, PA 2-6, secretes an antibody which binds to HLA-A, -B, and -C chains¹³ and precipitates a molecule of two polypeptides of apparent molecular weight 43 000 and 12 000 daltons.¹⁴

Normal tissues were obtained within 24 h of death from a girl (15 yr) who died of acute viral pneumonia and a man (65 yr) who died of a cerebro-vascular accident. Thymus was obtained within 24 h of delivery from a 34 wk stillborn fetus. Breast tissue was obtained from biopsies submitted for routine frozen section diagnosis, and additional non-malignant breast tissue was obtained from necropsies within 24 h of death.

These tissues (approximately 1 cm³) were snap-frozen at -70°C and sections cut either immediately or after storage at -20°C for up to two months. Additional samples of morphologically normal gastric mucosa and hepatic tissue were snap-frozen within 10 min of biopsy, and portions of a normal unused donor kidney were snap-frozen within 24 h of removal. These gastric, hepatic, and renal samples were stored in liquid nitrogen until tested. 5 µm frozen sections were cut, from all of these tissues, air-dried at 37°C for 1 h, or room temperature for 24 h, then washed three times in phosphate-buffered saline (PBS) pH 7.4. The monoclonal antibody from ascitic fluid diluted 1/1000 in PBS, was applied to the sections for 1 h at room temperature, after which the sections were washed three times in PBS. Rabbit antimouse IgG conjugated with fluorescein (Miles Yeda Ltd), diluted 1/30 in PBS, was then applied for 30 min at room temperature. The sections were washed a further three times in PBS, then mounted in glycerol:PBS (7:3 vol/vol), and examined by UV epi-illumination.

Control sections were incubated either with the fluorescein conjugated antibody only or with three

"substitute" mouse monoclonal antibodies against Mallory body protein (as found in liver disease); the tissue reactivity of the latter three antibodies is distinctive.¹⁵

Results

Investigation of the expression of HLA class 1 antigens in normal tissues showed that they were found consistently in only three groups of cells, namely lymphoid cells, certain epithelial cells, and lining cells of small vessels (Table 1).

Although lymphoid cells were always positive, there was some variation in the intensity of staining of HLA class 1 antigens in different lymphoid tissues. Lymphoid tissue of the spleen, tonsil, and lymph node stained uniformly, while in the thymus the medullary cells reacted much more strongly with antibody than cortical cells (Fig. 1).

HLA class 1 antigens were detected in the surface and glandular epithelium of both the skin and the mucous membranes of the gastrointestinal, respiratory and urogenital systems. The cells of the convoluted tubules of kidney also contained HLA class 1 antigens as did glomeruli (Fig. 2) but the staining of the former was unusual for two reasons. Firstly, the intensity was low, presumably reflecting a low density of HLA antigen molecules, and secondly it appeared that the cytoplasm reacted with the antibody. Furthermore, renal tubular and gastric mucosal cells only stained in fresh frozen biopsy material and not in necropsy material.

The third group in which HLA class 1 antigen could be detected was the lining cells of small vessels (Fig. 3). These were positive in all the tissues examined, and were often the most strongly staining cells, being comparable in intensity with lymphoid cells. Although in many tissues the vessels were

Table 1 *Distribution of HLA class 1 antigen in normal tissue**

Tissue	Positive cells*
Lymphoreticular system (thymus, spleen, lymph node, tonsil)	All lymphoid cells
Gastrointestinal system (tongue, oesophagus, stomach, pancreas, small and large intestine)	
Respiratory system (larynx, trachea, bronchus, lung)	All epithelial cells of surface and glands
Skin	
Breast	
Cardiovascular system (heart, aorta, common carotid artery)	Endothelial cells of small vessels
Urogenital system (kidney, bladder, ovary, testis)	Glomeruli; transitional epithelial cells and cytoplasm of renal tubular cells
Liver	Biliary epithelial cells and cytoplasm of sinusoidal lining cells
Central nervous system (motor cortex, cerebellum, spinal cord)	None
Skeletal system (smooth and voluntary muscle, fibrous, and adipose tissue)	Fibroblasts and cytoplasm of occasional smooth muscle fibres
Endocrine system (thyroid, adrenal, islet of Langerhans)	None

*In addition to the positive cells listed, the lining cells of small vessels were positive in all tissues. Unless otherwise stated, the positive staining involved only the cell surface.

Fig. 1 *Thymus. Medullary thymocytes (large arrows) react more strongly with monoclonal antibody to HLA (class I) antigens than cortical thymocytes (small arrows) $\times 600$.*

undoubtedly vascular (capillaries, arterioles, or venules), they could have been lymphatic in some tissues. In liver there was a continuous layer of staining along all sinusoids and it was impossible with the light microscope to distinguish between endothelial and Kupffer cells (Fig. 4). The staining of the sinusoidal lining cells of the liver, like renal tubular cells, was cytoplasmic and not restricted to the cell surface. In contrast the endothelium of the large blood vessels examined (aorta, common carotid artery) did not stain.

The only other positive cells were found between collagen bundles, and although it was impossible to identify them positively, they were presumed to be

fibroblasts. Occasionally rather ill-defined staining inside smooth muscle fibres was seen, but this was inconsistent and its significance is not clear. Accordingly, fibroblasts and smooth muscle cells have not definitely been categorised as expressing HLA class I antigens. These antigens were not detectable on any other cells of the normal tissues examined. In particular, hepatocytes (Fig. 4) and the parenchymal cells of the central nervous system (Fig. 3) were not stained. All of the sections incubated with the fluorescein conjugated antibody alone were also negative. The mouse monoclonal antibodies against Mallory body protein reacted with entirely different cell types¹⁵ from those which were positive with

Fig. 2 *Kidney. The glomeruli are strikingly positive. The cytoplasm of the renal tubules is less intensely stained (arrows) $\times 450$.*

Fig. 3 Brain. The parenchymal cells are unstained while the lining cells of small vessels react strongly with antibody $\times 450$.

Fig. 4 Liver. Sinusoidal lining cells contain HLA class 1 antigens but these are not detectable in normal hepatocytes; the granular material in hepatocytes (arrows) is autofluorescent lipofuscin $\times 400$.

monoclonal antibody against HLA class 1 antigens.

Table 2 shows the detection rate of HLA class 1 antigens in both malignant and non-malignant breast epithelium. In 13 out of 14 normal or non-malignant

Table 2 Detection of HLA class 1 antigens in mammary epithelium

Breast condition	No of cases	HLA positive*
Non malignant†	14	13
Malignant‡	17	9

*A positive result was recorded when virtually all of the cells in the tissue reacted with antibody against HLA class 1 antigens. A negative result indicates that less than 25% of the cells stained with antibody. Many of the positive tumour cells stained much less intensely than non-malignant epithelium.

†This group comprised two morphologically normal breasts and the remainder showed varying degrees of mastopathy.

‡This group was composed of extraductal invasive cancers of breast

cases examined, all ductal epithelium stained intensely and uniformly (Fig. 5) in the remaining biopsy, from a case of florid adenosis, less than 25% of mammary ducts were positive. In contrast, epithelium of breast cancers varied markedly in its staining. In 9 out of 17 tumours, virtually all of the cells stained with varying degrees of intensity (Fig. 5) while in the others (8 out of 17) virtually all of the cells were negative (Fig. 6).

Discussion

These results show that the tissue distribution of HLA class 1 antigens is not uniform. Antigens were detected in only three groups of cells, namely lymphoid cells, certain epithelial cells, and the lining of small vessels. These results contrast with the assumption, based on absorption experiments,^{2,3} that virtually all nucleated cells express HLA antigens.

There are several possible explanations for this apparent discrepancy.

(i) It is possible that the lack of staining in some normal and malignant cells may have been due to the failure of the antibody to detect certain HLA-A, -B and -C groups. However, we also tested kidney and two malignant and non-malignant breasts with two other monoclonal antibodies, one of which (W6/32) reacts with all known HLA-A, -B, and -C heavy chains¹⁶ and the other (PA 2-12)¹⁵ specific for β_2 microglobulin.¹⁵ These antibodies gave the same distribution of immunofluorescence in these organs as the monoclonal antibody (PA 2-6) used throughout this study. Therefore it seems likely that the negative cells reported have either no HLA-A, -B, -C antigens or very low concentrations which were undetectable in this assay. In the latter context, it should be noted that cortical thymocytes which were positive in this assay express concentrations of HLA class 1 antigens that are only just detectable by the fluorescence-activated cell sorter (or 5000 molecules/cell).¹⁴

(ii) Since most of the normal tissues examined were obtained at necropsy, autolysis may have

Fig. 5 Breast. Malignant breast epithelium (large arrows) and adjacent normal breast (small arrows) stain intensely with antibody to HLA class 1 antigens $\times 400$.

Fig. 6 Breast. The cells in this breast cancer (C) do not react with antibody to HLA class 1 antigens while the surrounding stroma contains positively reacting cells (arrows) $\times 600$.

accounted for a loss of HLA antigens; this was noted in stomach and renal tubular cells. However, examination of fresh-frozen biopsy specimens of liver and breast confirmed the observations made on necropsy tissue from these organs.

(iii) Failure to detect HLA antigens on some tumour cells could be due to masking by blocking agents;^{12 17} it has recently been shown that H-2 antigens on murine myeloma cells in ascitic fluid are not accessible to anti-H-2 antibody because they are

masked by a glycoprotein.¹⁷ Alternatively, the tumour cells may be expressing HLA antigens which are somewhat similar to those of normal cells—alien histocompatibility antigens;¹⁸ the latter may not react with the antibodies used here.

(iv) Further explanations for the apparent discrepancies between our findings and those of others may be due to methodological differences. Previous investigations have examined distribution of HLA antigens by absorbing anti-HLA antibodies with whole tissue homogenates.² Since these homogenates include vascular lining cells, which were HLA class 1 antigen-positive in this study, then these cells would absorb HLA antibody, giving an apparently positive result for the parenchymal cells of the tissue homogenate examined. It is of interest that Williams *et al.*,³ showed that the concentration of HLA class 1 antigens in liver and brain were 9% and 1% respectively of that found in spleen. The use of monoclonal antibody markedly reduces the possibility of false-positive reactions which can occur readily with polyclonal antisera.

The apparent detection of HLA class 1 antigens in the cytoplasm of at least two cell types, namely renal tubular cells and hepatic sinusoidal lining cells, is at variance with the current convention which assigns HLA antigens to the cell surface. Given that the material labelling is whole HLA class 1 antigen, there are several possible explanations. The staining may represent the presence of true cytoplasmic antigens, or perhaps more likely, it represents antigens bound to infoldings of the plasma membrane which occurs extensively in renal tubular epithelium.¹⁹ Alternatively, the antibody may bind to cytoplasmic HLA

antigens which have been absorbed or phagocytosed.

The selective distribution of HLA-A, -B, -C antigens may have important implications for host responses to viral infections and neoplasia. As mentioned in the introduction, cytotoxic T lymphocytes only attack foreign antigens when these are expressed on cells which also express HLA antigens in common with T cells.⁷ Thus, according to this hypothesis, viral infection of the HLA class I antigen-negative cells would have to be eliminated by immune mechanisms other than T cell cytotoxicity, or by T cell cytotoxicity based on another antigen system; unless, of course, viral infection induces HLA antigen expression. The relative efficiency of different types of immune response in handling virus infections *in vivo* is not clear. It may be significant that several diseases, which seem to involve an abnormal immune response to virus infection, are those where the virus target cells have undetectable concentrations of HLA-A, -B, -C antigens on their surface—for example, chronic active hepatitis after hepatitis B virus infection, and sub-acute sclerosing encephalitis after measles infection. Moreover, if MHC-restricted T cell cytotoxicity is involved in modulating growth of human breast cancers, this must vary from tumour to tumour assuming that it is HLA class I dependant. It will be of interest therefore to correlate prognosis with HLA detection in these and other human cancers.

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